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Short communication

EST-PCR markers developed for highbush blueberry are also useful for genetic fingerprinting and relationship studies in rabbiteye blueberry

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ABSTRACT

The pedigrees of most rabbiteye blueberry (*Vaccinium virgatum*) cultivars can be traced back to four wild selections, 'Ethel', 'Clara', 'Myers', and 'Black Giant'; thus, they result from a very narrow germplasm base and are highly related. Until now randomly amplified polymorphic DNA (RAPD) has been the only type of molecular marker used in rabbiteye blueberry. Here we have tested whether a type of sequence-tagged site (STS) marker which utilizes specific ~20-mer primers from expressed sequence tags (ESTs) of highbush blueberry (*V. corymbosum*), called EST-PCR markers, are useful for genetic fingerprinting and relationship studies in rabbiteye blueberry. Of 44 EST-PCR primer pairs, from an assortment of genes expressed in flower buds of cold acclimated and non-acclimated plants, and shown to amplify polymorphic fragments among a collection of highbush genotypes, 40 (91%) resulted in successful amplification, and 33 of those (83%) amplified polymorphic fragments among the rabbiteye genotypes. The average number of scorable bands per primer pair was two. A dendrogram constructed from genetic similarity values, based on the EST-PCR marker data, tended to group siblings and parent/progeny together, generally agreeing with pedigree information. A group of 20 markers from five EST-PCR primer pairs distinguished all the genotypes in this study. These markers are as easy to generate and as affordable as RAPDs, but are based on actual gene sequences, and should have general utility for DNA fingerprinting, genetic diversity, and mapping studies.

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1. Introduction

Commercial production of blueberry utilizes multiple species in the section *Cyanococcus* of the genus *Vaccinium*. About two-thirds of blueberry production is from improved cultivars mainly of *V. corymbosum* L. (tetraploid highbush blueberry) and its hybrids and, to a lesser extent, *V. virgatum* Ait. (hexaploid rabbiteye blueberry). The other one-third of blueberry production is from wild, managed stands of *V. angustifolium* Ait. (tetraploid lowbush blueberry) (USDA Statistics, http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1113).

Not as widely grown as highbush and lowbush blueberry, the rabbiteye blueberry's natural range is the southeastern U.S., encompassing northern Florida, southern Georgia, and southern Alabama (Brightwell et al., 1955). Efforts to domesticate rabbiteye blueberry began around 1893 with the transplantation of native seedlings by M.A. Sapp to his farm in northwestern Florida (Hancock and Draper, 1989; Ballington, 2001). Rabbiteye blueberry breeding began in 1939 in Tifton, Georgia, at the Coastal Plain Experiment Station. Since then, breeding programs in Georgia, Florida, and North Carolina, in collaboration with the USDA/ARS, have all worked to develop new, improved cultivars (Austin, 1979). Rabbiteye blueberry is traditionally grown commercially in the southern regions of the U.S. However, because it is vigorous, high-yielding, and adaptable to upland soils, breeding efforts to develop northern-adapted rabbiteye cultivars are currently underway (Ehlenfeldt et al., 2007).

Although wild populations of rabbiteye blueberry contain much genetic diversity (Ballington et al., 1984), most current cultivars result from a very narrow germplasm base, and thus, are highly related. The pedigrees of most, but not all, rabbiteye cultivars can be traced back to four wild selections, 'Ethel' (thought to be identical to 'Satilla') from southeastern Georgia, 'Clara' and 'Myers' from north-central Florida, and 'Black Giant' from western Florida (Lyrene, 1981; Aruna et al., 1993). As in highbush and lowbush blueberry, inbreeding depression is a problem in progeny from self crosses and crosses of closely related genotypes of rabbiteye blueberry, resulting in reduced fruit set, smaller berries, later-

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maturing berries (Meader and Darrow, 1944; Hellman and Moore, 1983), and reduced seedling survival and vigor (Lyrene, 1983). The use of molecular markers to measure genetic relatedness among cultivars and selections, and identify more diverse germplasm to use in breeding is a recognized approach for widening the germplasm base of cultivated genotypes (Aruna et al., 1993).

Until now, the only type of molecular marker used extensively in rabbiteye blueberry has been randomly amplified polymorphic DNA (RAPD) markers. Aruna et al. (1993) used RAPD markers first to investigate the extent of genetic relatedness among 19 cultivars of rabbiteye blueberry, 15 improved cultivars, and the four original selections from the wild mentioned previously. As expected, results showed that the improved cultivars are progressing towards increased genetic similarity when compared with the four wild selections. Later, Aruna et al. (1995) developed a cultivar key for distinguishing the 19 rabbiteye cultivars based on 11 RAPD markers amplified from four RAPD primers.

RAPD markers have since been criticized for being difficult to reproduce between laboratories because of the need to duplicate the exact conditions for reproducible amplification from the 10-mer random-sequence primers (Jones et al., 1997). This has led many researchers to look for more robust marker systems to use, such as sequence-tagged site (STS) markers that utilize specific ~20-mer primers from sequenced DNA. We have developed an expressed sequence tag (EST) database comprised of about 5000 ESTs from flower bud cDNA libraries from the highbush blueberry cultivar Bluecrop (Dhanaraj et al., 2004, 2007; GenBank link: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucest&cmd=search&term=vaccinium). In our initial efforts to develop markers from these sequences, we designed 30 PCR primer pairs from these ESTs and tested them in amplification reactions with genomic DNA from a collection of 15 highbush or highbush hybrid cultivars (Rowland et al., 2003b). Primers were designed near the ends of the ESTs to amplify as much of each gene as possible, to increase chances of detecting polymorphisms. Fifteen of the 30 primer pairs resulted in amplification of polymorphic fragments that were detectable directly after ethidium bromide staining of agarose gels. We are using these markers to further saturate a genetic linkage map of a diploid blueberry population (Rowland et al., 2003a). We have also shown that these highbush-derived EST-PCR markers are suitable for genetic relationship studies on wild lowbush blueberry (Bell et al., 2008). EST-based PCR markers have been developed for other plants as well, including Norway spruce (Schubert et al., 2001), sugi (Tsumura et al., 1997), and rhododendron (Wei et al., 2005).

Here we have tested the highbush-derived EST-PCR markers for their efficacy at distinguishing a collection of 28 rabbiteye cultivars and selections (many of which are closely related) and one highbush cultivar (included as a positive control and expected outlier). A dendrogram was constructed based on genetic similarity values calculated from number of shared bands for each pair of genotypes. In addition, the correlation between similarity coefficients, calculated from molecular marker data, and coefficients of coancestry, calculated from pedigree information, was evaluated.

2. Materials and methods

2.1. Plant material

Twenty-nine blueberry genotypes, including 28 rabbiteye or rabbiteye/highbush hybrid cultivars and selections and one highbush cultivar, were evaluated in this study. The highbush cultivar used was 'Bluecrop' because it is the one from which all the currently available blueberry ESTs were derived. Thus, 'Bluecrop' could serve as a positive control in the PCRs and as an outlier in the genetic relationship studies. Genotypes were maintained by the USDA/ARS (Blueberry and Cranberry Research Center, Chatsworth, NJ). The cultivars used are listed below [along with their parents]: Bluecrop [GM-37 (Jersey × Pioneer) × CU-5 (Stanley × June)], Aliceblue [Beckyblue O.P.], Austin [T-110 (Woodard × Garden Blue) × Brightwell], Baldwin [Tifblue × GA 6-40 (Myers × Black Giant)], Beckyblue [Fla 6-138 (*V. virgatum*, 6×) × E 96 (*V. corymbosum*, 4×)], Black Giant [native selection], Bluegem [Tifton 31 (Ethel × Callaway) O.P.], Bonita [Beckyblue O.P.], Brightwell [Tifblue × Menditoo], Callaway [Myers × Black Giant], Centurion [W-4 (native selection) × Callaway], Chaucer [Beckyblue O.P.], Choice [Tifton 31 (Ethel × Callaway) O.P.], Clara [native selection], Climax [Callaway × Ethel], Coastal [Myers × Black Giant], Delite [Bluebelle × T-15 [GA 10-144 (Myers × Black Giant) × W-8 (native selection)]], Ethel [native selection], Ira [Centurion × NC 911 (Tifblue × Menditoo)], Montgomery [NC 763 [GA 11-180 (Myers × Black Giant) × W-4 (native selection)] × Premier], Myers [native selection], Powderblue [Tifblue × Menditoo], Premier [Tifblue × Homebell], Satilla (=Ethel) [native selection], Snowflake [Fla K (Beckyblue O.P.) × NC 1830 (NC 7-63-3a *V. constablaei* × Premier)], Tifblue [Ethel × Clara], Windy [Fla 79-17 [Bluebelle × Fla M (Beckyblue O.P.)] × Fla 79-27 (pedigree lost)], Woodard [Ethel × Callaway], and Yadkin [Premier × Centurion].

2.2. Genomic DNA extraction

Young leaves were collected from field-grown plants of all the genotypes used in this study, ground with dry ice in a coffee grinder, and stored at −80 °C. DNA was extracted from leaf tissue (~5 g) using the CTAB procedure of Doyle and Doyle (1990) and quantified.

2.3. Generation of EST-PCR markers

Expressed sequence tag-polymerase chain reaction (EST-PCR) markers were initially developed for use in commercial highbush blueberry (Rowland et al., 2003b). EST/cDNA libraries were derived from cold acclimated and non-acclimated floral buds of the highbush cultivar Bluecrop, and a contig analysis was performed to identify unique genes (Dhanaraj et al., 2004). In unrelated projects, our laboratory is attempting to identify genes that are important for cold acclimation in blueberry, and are using these markers to map genes associated with cold hardiness in a diploid mapping population; therefore, many of the genes are related to cold stress. To generate the ESTs from these libraries, in some cases, single-pass nucleotide sequencing was performed from both ends, 5′ and 3′, of the cDNA inserts. However, in most cases, sequencing was performed from only the 5′ end of the cDNA inserts. Primer pairs were designed from sequence data from contigs using the P3 website (http://frodo.wi.mit.edu/primer3/input.htm), to allow for amplification of as much of each gene as possible from the available sequences. For instance, forward and reverse primers were designed from sequences near the 5′ and 3′ ends, respectively, for those cDNA inserts for which sequences were obtained from both 5′ and 3′ ends. If sequence data were obtained from only one end of the cDNA, then both forward and reverse primers were designed as far apart as possible from only that one end. Location near the ends of the ESTs was the only criterion used.

DNA amplification reactions were performed as described previously (Levi et al., 1993) with minor modifications as described by Stommel et al. (1997). Briefly, amplification reactions were carried out at least twice in 25 μL volumes containing reaction buffer (20 mM NaCl, 50 mM Tris–HCl pH 9, 1% Triton-X-100, and 0.1% bovine serum albumin), 1.6 mM $MgCl_2$, 200 μM each of dATP, dCTP, dGTP, and dTTP, 0.1 μM each of the forward and reverse EST primers, 0.7 units *Taq* DNA polymerase (Promega, Madison, WI), and 25 ng template DNA. DNA was amplified in an MJ Research (Watertown, MA) PTC-100 thermal cycler, programmed for an ini-

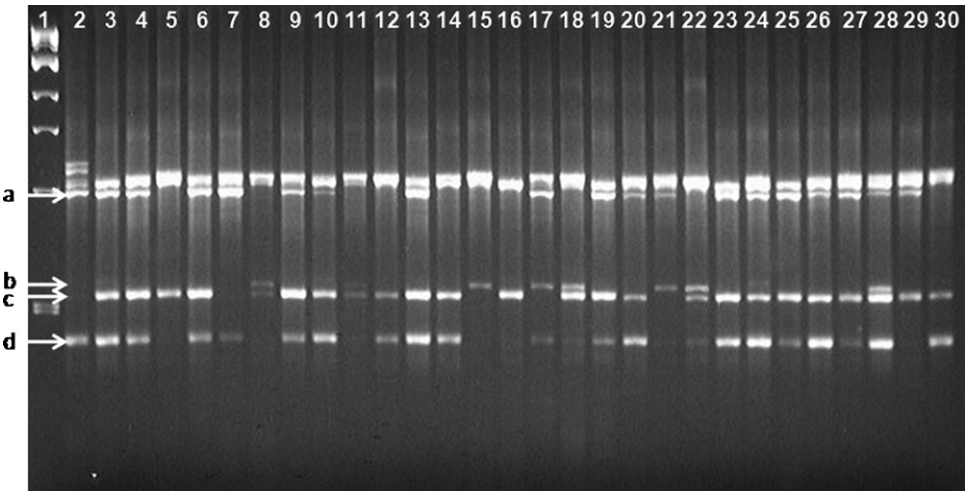

Fig. 1. Amplification products resulting from PCRs using forward and reverse primer pairs derived from EST CA287F and DNA from the 29 blueberry genotypes. Lanes 1–30 were loaded in the following order: (1) 1-kb ladder (Invitrogen Life Technologies, Carlsbad, CA), (2) 'Bluecrop', (3) 'Aliceblue', (4) 'Austin', (5) 'Baldwin', (6) 'Beckyblue', (7) 'Black Giant', (8) 'Bluegem', (9) 'Bonita', (10) 'Brightwell', (11) 'Callaway', (12) 'Centurion', (13) 'Chaucer', (14) 'Choice', (15) 'Clara', (16) 'Climax', (17) 'Coastal', (18) 'Delite', (19) 'Ethel', (20) 'Ira', (21) 'Montgomery', (22) 'Myers', (23) 'Powderblue', (24) 'Premier', (25) 'Satilla', (26) 'Snowflake', (27) 'Tifblue', (28) 'Windy', (29) 'Woodard', and (30) 'Yadkin'.

tial 5 min denaturation step at 95 °C, followed by 40 cycles of a 40 s denaturation step at 92 °C, 70 s annealing step at the appropriate annealing temperature, and 120 s extension step at 72 °C, and finally a 10 min extension step at 72 °C. The annealing temperature for each primer was calculated as (4 °C × # of Gs and Cs) + (2 °C × # of As and Ts), and the lower of the two possible annealing temperatures was used for the reaction. Amplification products were separated by electrophoresis through 1.4% agarose gels containing 0.5 μg· mL^{-1} ethidium bromide.

2.4. Marker data analysis

Strong, reproducible fragments were scored for their absence or presence. The Numerical Taxonomy and Multivariate Analysis System program package for PC (NTSYS-pc, version 2.1, Exeter Software, Setauket, NY) was used to construct a similarity matrix from the EST-PCR data (based on the Dice coefficient (Dice, 1945)) and to perform a cluster analysis of the resulting similarity matrix. A dendrogram of the genotypes was constructed by applying the unweighted pair-group clustering method (UPGMA) to the genetic similarity matrix. A cophenetic value matrix was produced from the tree matrix to measure the goodness of fit of the tree to the genetic similarity matrix on which it was based.

Coefficients of coancestry were determined from pedigree information using SAS Procedure INBREED by calculating the inbreeding coefficients of offspring from the hypothetical mating of each pair of blueberry genotypes, assuming disomic inheritance. To determine the level of correlation between the genetic similarity values derived from the molecular marker data and the pairwise coefficients of coancestry, Pearson product–moment correlation coefficients were calculated using SAS Procedure CORR.

3. Results and discussion

3.1. Development and testing of EST-PCR markers in rabbiteye blueberry

The EST-PCR primer pairs, designed from the ends of the available nucleotide sequences from the highbush cultivar Bluecrop, were tested in amplification reactions with DNA from 28 rabbiteye or rabbiteye/highbush hybrid cultivars and selections and one highbush cultivar, Bluecrop itself. The cDNA clones, from which the ESTs were derived, encoded a wide range of proteins including temperature stress-related proteins (DNA J, low temperature-induced 65 kDa protein, dehydrin, late embryogenesis abundant protein, early light-induced protein), proteins involved in signal transduction (calmodulin-binding protein, serine/threonine protein kinase), and basic metabolic proteins (beta amylase, sucrose synthase, aldehyde dehydrogenase, cinnamyl alcohol dehydrogenase, pyruvate decarboxylase), among others (data not shown).

A total of 44 primer pairs were tested in PCRs with the rabbiteye genotypes. These were a subset of primer pairs known to amplify polymorphic fragments on a collection of highbush genotypes. Annealing temperatures ranged from 52 °C to 62 °C. Of the 44 primer pairs tested on the rabbiteye genotypes, 40 (91%) resulted in successful amplification, and 33 of those (83%) amplified polymorphic fragments among the genotypes. Amplification profiles of PCRs using EST primer pairs from CA287 are shown in Fig. 1.

A total of 54 polymorphic fragments from 24 primer pairs (Table 1) were chosen for scoring, based on their clarity, size separation, and reproducibility. The number of scored bands ranged from one to six, and averaged two per primer pair. All genotypes were distinguishable from each other, with the exception of 'Ethel' and 'Satilla'. Although maintained as two separate selections, these have been considered by some breeders to be genetically identical (Austin, 1984). Also, Aruna et al. (1995) concluded that 'Ethel' and 'Satilla' are likely identical, based on 186 RAPD fragments generated from 19 primers. From the 54 polymorphic EST-PCR fragments, subsets of scorable markers that were sufficient for all the possible distinctions could be identified. One such subset included amplification products from five EST-PCR primer pairs, CA21, CA34, CA148, CA287, and NA11.

3.2. Genetic similarity coefficients and genetic relatedness trees

Similarity coefficients based on the 54 polymorphic EST-PCR fragments were calculated for each pair of genotypes. Excluding the comparison of 'Ethel' and 'Satilla', which appeared identical, similarity values ranged from 0.216 (for 'Baldwin' and 'Bluecrop' with no parents in common) to 0.889 (for 'Premier' and 'Yadkin', where 'Premier' is a parent of 'Yadkin'). The average similarity value among all the rabbiteye genotype comparisons, excluding again those that were identical, was 0.605, an increase over the average

Table 1
Highbush-derived EST-PCR primer pairs found to amplify polymorphic-sized fragments among the tested rabbiteye genotypes. Only those used in the genetic relationship study are shown.

Sequence name/accession number	Primer sequences (5′–3′)	Annealing temperature (°C)
CA4F/CF810760	GCC GCT CTT CTC TTC CTA GC	58
CA4R/CF811108	CCA TCA AAC ACC ACC TAT GC	
CA15F/CF810480	CTA GAG GCT GCA GTG GAA GC	58
CA15R/CF811066	TTG CTC GTG TCG TCC TTA TG	
CA21F/CF810526	TCC GAT AAC CGT TAC CAA GC	56
CA21R/CF811092	TAT ACA GCG ACA CGC CAA AA	
CA23F/CF810543	GTT AGA GAG GGT TTC GAG GA	54
CA23R/CF811093	AGC AAA AAC TTC ACG CCA AT	
CA31F/CF810617	AGC ATT TGA CAC CAG TCA CG	52
	TTA CAG GAG GGG GAT TTT	
CA34F/CF810644	CTA AAG ACG GGC CTG AAG TG	60
	TCT GGT GAG AAC TGG TCG TG	
CA39F/CF810685	TAA TGA GTC TGT GGC GAA CG	56
CA39R/CF811100	AAC AAG ACC AAA CCC CAC AT	
CA43F/CF810721	ACA TGG GTG GTC AAG GTC AT	58
CA43R/CF811102	GTC TAC ATG CCA CCG TCC TC	
CA45F/CF810736	AGA GAC TGC TGC TGG TGA CA	60
CA45R/CF811104	CGC ACG TAC TTG GCT ATC AG	
CA47F/CF810751	CTG CTG ATC CTA GCC ACC TC	58
CA47R/CF811106	AAA GGT TGC CCA AAA GTT CC	
CA51F/NA[a]	GCT GCT CTT GTA CAG GGC TC	55
CA51R/NA	TTG CGC ACA CAT AAA CCT AAA	
CA106F/CF810439	TCA TGC CTT CTC TCG CTG TA	58
CA106R/CF811031	TGG CAA CAC AAA GGC TAG TG	
CA111F/CF810442	GAC CAA ACC GGA AGC TAC AC	56
CA111R/CF811034	GCA AGG GTC AAA ACG TGA AT	
CA148F/CF810470	CAA GGG TGC ACG TGA ACT TA	56
CA148R/CF811057	AAA AGC ATT GCA GTC ACA CG	
CA287F/CF810588	AGG GCT TTC CCT CAA TCA CT	58
	CCT TGT TGT TCC TTC CTT CG	
CA570F/CF810815	ACA GCA CCA GAG GGA GAG AA	60
	CGG CCG AAG AAT ACA CAT CT	
CA1440F/CV090457	GAG GCG CTA CAA GCA GAA AC	60
	CAT CAT CGT CAT CGT CAT CC	
NA5F/CF811449	GCC ATG GTG GAA TGA GTT GT	56
	TTT TTC AGC AAA TGG AAA TTC A	
NA9F/CF811668	CGC TGG TTA GCC TTT TTG AG	60
	CTG GAC TCA ATG GCA CAA GA	
NA11F/CF811182	GGA AAT GCT GCC CAC TGT AT	54
	CAA ATT TGC CAG CAA AAC C	
NA13F/CF811202	TTA CTC GCC GAA GCT CAA AT	58
	GGC GAA ACC ATC ACT TTC TC	
NA27F/CF811332	CGC TCG CTC CAT TGT TTC	56
	TAT GCA TGA AGC TTG CCG TA	
NA95F/CF811673	GGT GCA TTG GGT TTC AGC TA	60
	TTT TTG AGA GAG ATT GCC ATG TA	
NA1063F/CV090899	TTT TCC GAG TGG TGG TTA GC	60
NA1063R/CV091499	ATA ACC AGC CAC GCT TTC TC	

[a] NA – not applicable. Sequences were not deposited in GenBank because they had too many Ns.

similarity value of 0.517 among the wild parental selections ('Black Giant', 'Clara', 'Ethel', and 'Myers'). Likewise, Aruna et al. (1993), using RAPD markers, found that genetic similarity was increased among the improved cultivars relative to the four parental selections. Of all the genotypes, the most divergent was the highbush cultivar Bluecrop, as expected, with an average pairwise similarity value of 0.462. Of the rabbiteye genotypes, the most divergent was the wild selection 'Black Giant', with an average pairwise similarity value of 0.483. The least divergent was 'Yadkin', with an average pairwise similarity value of 0.677.

The dendrogram, based on a cluster analysis of the similarity matrix, is shown in Fig. 2. A cophenetic value matrix was produced from the tree matrix to measure the goodness of fit to the similarity matrix. An *r* value of 0.835 indicated a very good fit.

As expected, 'Bluecrop' was the outlier in the tree. The closer groupings on the tree tended to be between immediate parents and their progeny and full and half sibs. The first division of the rabbiteye genotypes was at about 52% similarity. One of the two groups resulting from this separation included three of the parental selections, 'Black Giant', 'Clara', and 'Myers', whereas the other group included the parental selection 'Ethel' (or 'Satilla'). Within the smaller group (comprised of 'Black Giant', 'Clara', and 'Myers', along with eight other genotypes) was a subgroup that included 'Black Giant', 'Coastal', 'Callaway', 'Centurion', 'Climax', and 'Delite'. Pedigrees indicate that 'Black Giant' is a parent of 'Coastal' and 'Callaway', and 'Callaway' is a parent of 'Centurion' and 'Climax' and grandparent of 'Delite'. In fact, 'Coastal' and 'Callaway' are full sibs, being progeny of 'Black Giant' and 'Myers'.

Within the larger group (comprised of 'Ethel' and 15 other genotypes), there were also many subgroups that agreed with the parentage of the genotypes. For example, 'Beckyblue' grouped with 'Bonita', for which it is a parent. Within this larger group were also 'Aliceblue' and 'Chaucer', both of which have 'Beckyblue' as a parent, and thus are half sibs to 'Bonita'. Also, 'Yadkin' and 'Premier' (which is a parent of 'Yadkin') grouped together. Other genotypes within this larger group were 'Brightwell', 'Austin', 'Tifblue', 'Powderblue', 'Ethel', 'Woodard', and 'Choice', for which close relationships are known. 'Brightwell' is a parent of 'Austin'. 'Tifblue' is a parent of 'Brightwell', 'Premier', and 'Powderblue'; and 'Ethel' is a parent of 'Tifblue' and 'Woodard' and grandparent of 'Choice'.

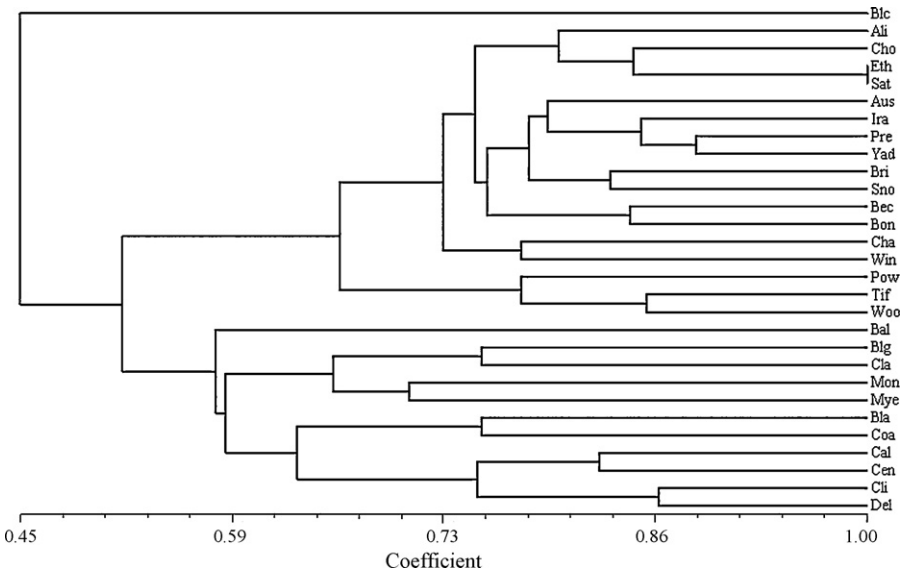

Fig. 2. Dendrogram of 29 blueberry genotypes (1 highbush, 28 rabbiteye) generated by UPGMA cluster analysis of the Dice similarity matrix based on EST-PCR markers. Genotypes are designated by the following abbreviations: 'Bluecrop' (Blc), 'Aliceblue' (Ali), 'Austin' (Aus), 'Baldwin' (Bal), 'Beckyblue' (Bec), 'Black Giant' (Bla), 'Bluegem' (Blg), 'Bonita' (Bon), 'Brightwell' (Bri), 'Callaway' (Cal), 'Centurion' (Cen), 'Chaucer' (Cha), 'Choice' (Cho), 'Clara' (Cla), 'Climax' (Cli), 'Coastal' (Coa), 'Delite' (Del), 'Ethel' (Eth), 'Ira' (Ira), 'Montgomery' (Mon), 'Myers' (Mye), 'Powderblue' (Pow), 'Premier' (Pre), 'Satilla' (Sat), 'Snowflake' (Sno), 'Tifblue' (Tif), 'Windy' (Win), 'Woodard' (Woo), and 'Yadkin' (Yad).

Coefficients of coancestry were calculated for all possible pairs of the genotypes based on pedigree information. Coefficients of coancestry ranged from 0, for those genotypes with no ancestors in common, to 0.305, for 'Brightwell' and 'Austin', which are parent/progeny and have several other ancestors in common. The second highest coefficient of coancestry was 0.281 for several parent/progeny pairs, 'Premier'/'Yadkin', 'Centurion'/'Yadkin', and 'Premier'/'Montgomery'. A correlation test was performed on the genetic similarity matrix based on the molecular marker data and the coeffcient of coancestry matrix based on pedigree information. A fair positive correlation was found ($r = 0.3$), which was highly significant ($P \leq 0.0001$), and on the same order of magnitude as the correlations found between genetic similarity matrices based on molecular marker data and coefficient of coancestry matrices for a collection of predominantly northern highbush cultivars ($r = 0.3$; $P \leq 0.0001$) (Rowland et al., 2003b) and a collection of southern highbush cultivars ($r = 0.57$; $P \leq 0.0001$) (Brevis et al., 2008).

In conclusion, our highbush-derived EST-PCR markers proved very effective at distinguishing the 28 rabbiteye cultivars and selections used in this study. A dendrogram constructed from the genetic similarity values, agreed fairly well with pedigree information, tending to group siblings and parent/progeny together. This demonstrates that these markers are useful for DNA fingerprinting and assessing genetic relationships, and should be extremely useful for identifying more genetically diverse germplasm for incorporation into rabbiteye breeding programs. These markers are easy to generate and affordable for most labs, requiring only standard PCR and agarose gel electrophoresis equipment.

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